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Preparation, photoluminescence properties and application for *in vivo* tumor imaging of curcumin derivative-functionalized graphene oxide composite

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ABSTRACT

Curcumin derivative-functionalized graphene oxide (CUDE-f-GO) composite was synthesized via direct polycondensation reaction in a binary phase system. The composite was characterized by FTIR, ^{1}H NMR, Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), thermal gravimetric analysis (TGA) and atomic force microscopy (AFM). Digital camera and transmission electron microscopy (TEM) images showed that the composite had better dispersion in phosphate buffer solution (PBS) than graphene oxide (GO). The photophysical experiments revealed that the photoluminescence (PL) properties were significantly enhanced when curcumin derivative (CUDE) was covalently grafted on the surfaces of GO. The *in vitro* and *in vivo* bioimaging results showed that CUDE-f-GO composite was an ideal optical material for biological imaging because of its good photostability, low cytotoxicity, bright bioimaging and excellent tumor targeting ability.

1. Introduction

Biological imaging probes were widely used as tools in monitoring and recording molecular spatiotemporal distribution, cellular biochemistry processes, and even detecting earlier diseases [1–3]. Therefore, many researchers have been devoted to the development of new probes, such as multi-branched organic molecules, polymers, quantum dots, coordination compounds and so on, for the use in imaging [4–6]. Despite the efforts paid to prepare various bioimaging probes, an ideal biological fluorescence probe which can exactly and efficiently reflect the cell environment is very rare by limitations of autofluorescence from the biological samples, unstable fluorescent signal, biocompatibility and cytotoxicity, etc [7–9]. Moreover, the synthetic methods of multi-branched molecules with π-conjugated motifs by incorporating acceptor and donor moieties were suffered from the complex experimental procedures and low-yielding, which greatly limited their practical application.

Curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], which is obtained from the rhizome of Curcuma longa Linn, is a natural phenolic pigment with low toxicity and good stability and also a common ingredient used in cosmetics, spices and traditional chinese medicines in Asian countries [10,11]. The compound and its derivatives have been reported to possess good optical and electrical properties because of a highly π-electron delocalized system and symmetric structure [10,12]. However, their applications have been limited by their low potency and poor bioavailability.

Graphene oxide (GO) has a large number of carboxyl, hydroxyl and epoxy groups on its basal planes and edges, which endow GO with good water-solubility [13,14]. In addition, these oxygen-containing groups provide possibility for GO to be functionalized through covalent or noncovalent bonding modification [15,16]. Especially, no obvious toxic effects *in vivo* and the use as a specific delivery carrier of bioactive molecules or drugs had made GO a promising candidate for disease therapy or diagnostics applications

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[17].

A great deal of research displays that the functionalization of GO with small organic molecules not only prevents aggregation of GO, but also enhances the physical properties of GO [18,19]. As stated above and our previous work [20,21], in this study, a novel curcumin derivative-functionalized graphene oxide (CUDE-f-GO) composite with better dispersibility and optical response has been prepared via a facile grafting method. Furthermore, the cytoxicity, photophysical properties, *in vitro* and *in vivo* bioimaging of the composite were also investigated. The synthetic route of as-prerared CUDE-f-GO composite is presented in Scheme 1.

2. Materials and methods

2.1. Reagents

All solvents and starting materials were obtained from commercial suppliers and used without further purification. Solvents used in spectra experiments were spectrometric grade. Double distilled water was used throughout all experiments.

2.2. Preparation of CUDE-f-GO composite

Curcumin derivative (CUDE), 1,7-bis(4-bromobutyloxy-3-methoxy-phenyl)-1,6- heptadiene-3,5-dione, was synthesized as the method described by us before [22]. Graphite oxide (GO) was prepared by a modified Hummer's method through completely oxidizing graphite flakes in a solution of nitric acid, sulfuric acid and potassium chlorate for about 96 h [23].

Functionalization of GO by grafting CUDE chains was performed as follows [24]. GO (30 mg) was dissolved in 40 mL of dimethyl formamide (DMF) under sonication dispersion. The anhydrous potassium carbonate (1.06 g, 7.7 mmol) was added into the above solution, and refluxed continuously for 1 h. Then, CUDE (1.4 g, 2.2 mmol) and a small quantity of potassium iodide were added into the preceding reaction system. The reaction mixture was refluxed at 80 °C for about 6 h under vigorous stirring and monitored by thin-layer chromatography (TLC). After the completion of the reaction, the reacted solution was cooled, filtered and washed with dichloromethane 4 times. The product was dried in vacuum oven at 50 °C for 24 h.

2.3. Characterization of CUDE-f-GO composite

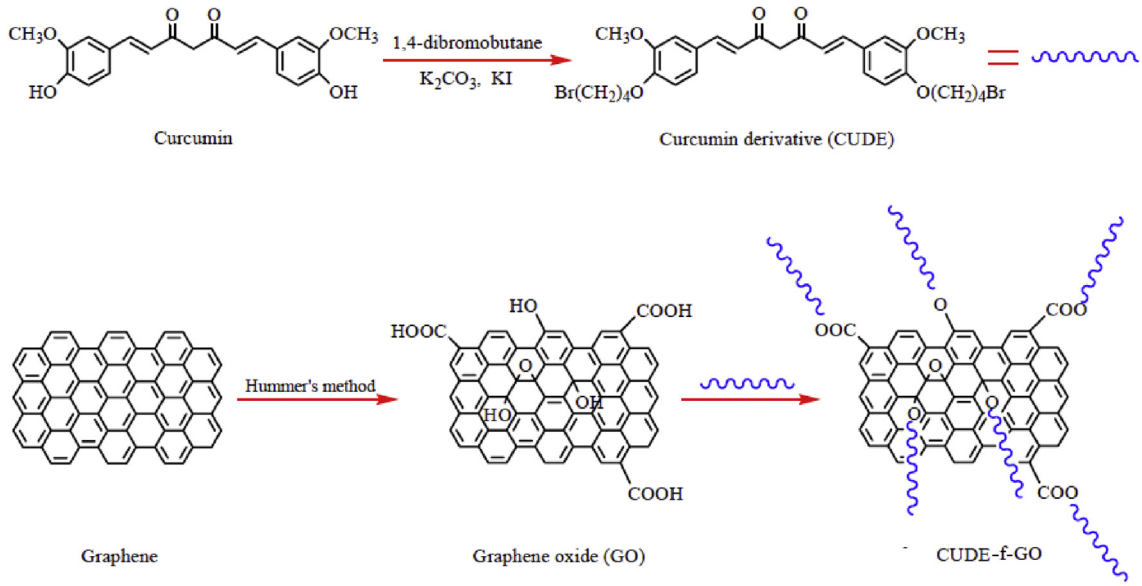
Fourier transform infrared (FTIR) spectra were recorded on a PE GX spectrometer (Perkin-Elmer, USA) at room temperature on KBr pellets with sample concentration of ~1% from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. ^1H NMR spectra were recorded on a BrukerDRX-400 NMR instrument using DMSO as the solvent. Atomic force microscopy (AFM) images were carried out on a Dimension Scanning Probe microscope (FemtoScan, Russia) under tapping mode. Raman spectra were taken by a Craic Tech spectrometer with laser excitation at 720 nm. The X-ray photoelectron spectroscopy (XPS) spectra were recorded on a Kratos AXIS Ultra X-ray photoelectron spectrometer. Thermal gravimetric analysis (TGA) spectra were carried out in a TGA PYRIS6 thermogravimetric analyzer (Perkin-Elmer, USA) using a heating rate of 10 °C/min from 25 to 700 °C in a nitrogen atmosphere.

2.4. Dispersion experiments

GO and CUDE-f-GO composite were dispersed in phosphate buffer solution (PBS, pH = 7.2, Gibco) and DMSO at a concentration of 50 μM, respectively. Suspensions were imaged using a digital camera after predetermined intervals. Transmission electron microscopy (TEM) images of the two compounds were also taken in PBS on a JEOL JEM1200EX electron microscope at 120 kV.

2.5. Optical properties studies

Ultraviolet–visible (UV–Vis) light spectra of the compounds in DMF with the concentration of 50 μM were achieved on a Perkin Elmer Lambda 900 spectrometer from 200 to 800 nm with a 1 nm resolution. Photoluminescence (PL) spectra were conducted at the same concentration using a Horiba Fluoromax 4 with excitation sources from 200 to 500 nm and emission readings from 400 to 700 nm with a wavelength resolution of 2 nm. The quartz cuvettes used had a 1 cm path length. The solvatochromic behaviors were also investigated at the same conditions with CUDE-f-GO composite in four different solvents (CH_2Cl_2, EtOH, DMF and DMSO),



Scheme 1. Synthesis routes of CUDE-f-GO composite.